IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Rozhon *et al.*	Confirmation No.:	9130
Serial No.:	09/712,033	Art Unit:	1651
Filed:	November 14, 2000	Examiner:	Irene Marx

For: ENTERIC FORMULATIONS OF PROANTHOCYANIDIN POLYMER ANTIDIARRHEAL COMPOSITIONS

Attorney Docket No.:
P&E Ref. 11133-004-999

JD Ref. 706788-999003

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Akram Sabouni, Ph.D., declare and state as follows:

1. I am a co-inventor of the above-identified application which I have read and understand.

2. My educational background and patents/patent applications of which I am an inventor are briefly described in Exhibit 1 attached to this Declaration. I received a Ph.D. in Pharmaceutics from the University of Bonn, West Germany in 1984 and have post-doctoral experience in Pharmaceutics and Drug Delivery from West Virginia University and the University of Cincinnati.

3. My professional experience includes twenty years experience in pharmaceutical research and development of a wide variety of small molecules, peptides, and protein drugs. I have been an author or co-author of more than 45 publications and presentations. I have served at a number of pharmaceutical companies as a research scientist, group leader and a Director of Product and Process Development and am currently Vice President, Product and Business Development.

4. At the time the application was filed I was an employee of Shaman Pharmaceuticals Inc., original assignee of this application. My position was as Group Leader and I was employed for about 3 ½ years at Shaman Pharmaceuticals. Currently, I am serving as a consultant to PS Pharmaceuticals, Inc., the current assignee.

5. I have reviewed the results of experiments presented in Section 6 of the above-identified application at pages 37-43. These experiments were conducted under my supervision to investigate whether or not gastric or intestinal fluid had any effect on the stability of isolated aqueous soluble proanthocyanidin polymer composition of Croton. The results obtained indicate that the isolated aqueous proanthocyanidin polymer composition is not stable in conditions which mimic those in the stomach following oral administration but is stable in conditions which mimic those in the intestine.

6. These results were particularly surprising and I recall discussions with colleagues at Shaman who were familiar with the isolated aqueous soluble Croton polymer who strongly expressed their surprise at the results. These unexpected results, however, were reproducible. Based, in part, on these unexpected results, my co-inventors and I conceived the present methods for orally administering the isolated aqueous soluble proanthocyanidin polymer composition in enterically protected form.

7. The following multiple experiments were conducted on behalf of me and my co-inventors or under my supervision while I was an employee of Shaman Pharmaceuticals Inc. As explained in detail below, the results obtained clearly demonstrate that, unexpectedly, only following enteric protection was the aqueous soluble proanthocyanidin polymer isolated from Croton species efficacious to treat secretory diarrhea in a relevant animal model.

8. Methods: Side-by-side experiments were performed using the murine cholera toxin (CT) treated model of secretory diarrhea described in Section 7 of the specification, at pages 44-47, using an isolated aqueous soluble proanthocyanidin polymer composition of Croton species (designated "SP-303") either enterically protected, e.g., by formulation with $NaHCO_3$ designated "SP-303+$NaHCO_3$" or without enteric protection, i.e., in water, designated "SP-303 no $NaHCO_3$." The experiments were performed as follows:

50- to 52-day-old mice with body masses that ranged from 15.7 to 18.7 g were used. Test animals were wild type C57B1/6 and were obtained from Charles River Lab. All animals were maintained in metabolism cages with water *ad libidum* for the duration of the experiment. Mice were fasted for 24 hours prior to start of the experiment and were deprived of food during the course of experimentation. Initially (t_0 h), mice were orally dosed with cholera toxin (CT (15μg)) and anorectally sealed with a cyano-acrylamide ester (Superglue). Three hours later (t_3 h), mice were orally dosed with an isolated, aqueous soluble, enterically protected proanthocyanidin polymer composition from Croton species (SP-303) (at a dose of 0.1, 0.3, 1.0, 3.0, 10, 25, 50 or 100 mg/kg in $NaHCO_3$) or SP-303 in water at 50 mg/kg.

Control animals were dosed (at t_3 h) with either: $NaHCO_3$ alone, $NaHCO_3$ + CT or H_2O. After a 6 (t_6 h) or 7 hour (t_7 h) incubation of CT, mice were sacrificed and the entire murine small intestine from the pylorus to the rectum including cecum was isolated. Care was taken to avoid tissue rupture and loss of fluid, and the attached mesentery and connective tissues were then removed. The mass of the tissue and the fluid within was determined using an analytical balance. The tissue was then opened longitudinally, the fluid removed, and the tissue was patted dry. Fluid accumulation was measured as ratio of the mass of accumulated fluid in the intestine (small and large including cecum) versus the mass of the intestine minus the mass of the fluid.

9. Results: The results of the experiments are presented in Table 1, Exhibit 2 attached to this Declaration.

10. As shown in Exhibit 2, administration of 25 mg/kg or more isolated proanthocyanidin polymer of Croton species formulated to be enterically protected (SP-303+$NaHCO_3$) significantly ($p<0.05$) reduced fluid accumulation in the murine model of secretory diarrhea compared to controls.

11. In complete contrast, when administered at a dose of 50 mg/kg without enteric protection (SP-303-no $NaHCO_3$), there was no significant reduction of fluid accumulation. Such results were completely unexpected.

12. I have also reviewed the results of experiments presented in the specification of this application, in particular in Section 7 at pages 44-50 and Section 9 at pages 59-62. The results of experiments presented in Section 7, particularly in Tables 4 and 5 (at pages 49 and 50, respectively) clearly support the unexpected results presented in Exhibit 2 and demonstrate that enterically protected isolated proanthocyanidin polymers from Croton formulated as beads coated with EUDRAGIT™ which resists stomach acids significantly reduced fluid accumulation in the murine cholera model of secretory diarrhea.

13. Results presented in Tables 7-9 at pages 60-62 demonstrate that enterically protected isolated aqueous soluble proanthocyanidin from Croton is useful to ameliorate stool frequency and gastrointestinal symptoms.

14. Based upon the results presented in Exhibit 2 and in the specification as detailed above, it is my opinion and judgment that enteric protection is needed for the isolated, aqueous soluble Croton proanthocyanidin polymers to be effective as anti-diarrheal agent. Such results were surprising and unexpected and would be so viewed by one skilled in the art.

I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: 7-2-04

A. Sabouni

Dr. Akram Sabouni

Exhibit 1

EDUCATION

January 1987 - December 1988
Post Doctoral Research Associate
Department of Pharmaceutics and Drug Delivery
College of Pharmacy, University of Cincinnati
Supervisor: Dr. Wolfgang Ritschel

September 1984 - December 1986
Post Doctoral Research Associate
School of Pharmacy, West Virginia University
Supervisor: Dr. Carl J. Malanga

September 1979 - March 1984
Ph. D. in Pharmaceutics
College of Pharmacy, University of Bonn, West Germany
Advisor: Dr. Gottfried Blaschke

June 1972 - June 1977
B.Sc. in Pharmacy
College of Pharmacy, University of Damascus

PATENTS

E. Rozhon, A. Khandwala, and **A. Sabouni**: Enteric Formulations of Proanthocyanidin Polymer Anti-diarrheal Compositions. 0935417/EP-A1, 1998

R. Hector and **A. Sabouni**: Methods and Composition for Treating Fungal Infections in Mammals, # 5789387,1998

S. Leung, Q. Ha, **A. Sabouni**, S. Gadiraju, Q. Ha, A. Ifan: Method for Manufacture of Extended Release Dosage Form, 20020192293/US-A1, 2002

EXHIBIT 2

Table 1. The effect of enteric protected SP-303 on intestinal fluid accumulation in CT-treated mice.

No. of Mice	Treatment	Fluid Accumulation* (mg fluid / mg intestine)
7	$NaHCO_3$ alone	0.69 ± 0.08
20	$NaHCO_3$ + CT	1.80 ± 0.77
6	0.1 mg/kg SP-303 + $NaHCO_3$	2.04 ± 1.09
6	0.3 mg/kg SP-303 + $NaHCO_3$	2.16 ± 0.93
6	1.0 mg/kg SP-303 + $NaHCO_3$	1.66 ± 0.30
5	3.0 mg/kg SP-303 + $NaHCO_3$	1.26 ± 1.44
6	10 mg/kg SP-303 + $NaHCO_3$	1.24 ± 0.57
10	25 mg/kg SP-303 + $NaHCO_3$	0.97 ± 0.35*
7	50 mg/kg SP-303 + $NaHCO_3$	0.73 ± 0.24*
12	100 mg/kg SP-303 + $NaHCO_3$	0.81 ± 0.26*
10	50 mg/kg SP-303 no $NaHCO_3$	1.36 ± 0.42
8	H_2O	1.28 ± 0.09 a

*Value significantly different from $NaHCO_3$+CT